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Spermatozoon ultrastructure in *Mecopoda nipponensis* (Othoptera: Tettigonioidea, Mecopodidae)

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Abstract: This paper describes the spermatozoon ultrastructure in *Mecopoda nipponensis* (De Haan). Spermatozoon of this species were examined by transmission electron microscopy after double fixation of live material in glutaric-aldehyde and osmic acid. Its acrosomal complex lies to the side of the flat, dense nucleus and partially embraces it. Two broad bean-shaped accessory bodies are located laterally to the axoneme, which is of a typical 9 + 9 + 2 pattern. Two almost completely crystallized mitochondrial derivatives are oval in cross-section. There are three connecting bands between mitochondrial derivatives and axoneme. Two flat membranous cisterns close to mitochondrial derivatives are S-shaped in cross-section. A character peculiar to this species, an additional bi-layer membrane, appears around organelles of the tail in some cross-sections.

Key words: Orthoptera; Mecopodidae; *Mecopoda nipponensis*; spermatozoon; ultrastructure

Spermatozoa, the germ cells of male animals, are highly specialized and play an important role in the reproductive isolation of species. Spermiogenesis and differentiation of spermatozoon are conspicuously different in various groups of insects, which reflects their different degrees of phylogenetic relatedness (Chung *et al*., 1982). Investigation of the spermatozoon ultrastructure can thus help resolve some unclear systematic relationships between taxa (Xi *et al*., 1995, 1997; Yin *et al*., 1992). Moreover, it can provide a theoretical basis for investigating the evolution of taxa (Yin *et al*., 1992; Dallai *et al*., 1983).

Baccetti (1970, 1979, 1987), Afzelius (1969) and Phillips (1969, 1970) have published many papers on the morphology and ultrastructure of spermatozoa, including those of insects. After long research on orthopteran taxonomy and sperm ultrastructure, Baccetti believes that spermatology can be of great help to phylogenetic reconstruction at any taxonomic level (Baccetti, 1987). Chung (1982) and Xi (1995, 1997, 1999) also did much work on spermiogenesis and the ultrastructure of spermatozoa in the Acridoidea and Grylloidea. However, the spermatozoa of the Mecopodidae of the Tettigonioidea have never been investigated. This paper describes the spermatozoon ultrastructure in *Mecopoda nipponensis* (De Haan).

1 Materials and Methods

Live adult males of *Mecopoda nipponensis* were collected from Bei-bei in Chongqing, China, Sept. 2001.

After dissection, testes of adult males were fixed in 2.5% glutaric-aldehyde solution in phosphate buffer (0.1M, pH 7.2) for 2 – 3 hours, and postfixed for 2 hours in 1% osmic acid in the same buffer. Thereafter, they were dehydrated with ethanol and embedded in Epon-168. The sections of testes were cut with a LKB-5 ultramicrotome and were then stained with lead acetate and lead citrate. Finally, the sections were examined

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with a JEM-2000EX electron microscope.

2 Results

The spermatozoon laminar acrosomal complex of *Mecopoda nipponensis* lies lateral to the flat, dense nucleus and partially embrances it. The flat acrosome, with two processes facing the nucleus, embraces the anterior part of the nucleus (Pl. Ⅰ: 1 – 2).

The nuclei of spermatozoa, being extremely dense, compact and devoid of visible substructure, appeard crescent-shaped and semilunar in cross-section (Pl. Ⅰ: 3). Its anterior part, which inserts laterally in the acrosomal complex, is mostly hat-shaped (Pl. Ⅰ: 1), and the part immediately separated from the acrosomal complex is crescent-shaped in cross-section (Pl. Ⅰ: 2).

The centriolar adjunct, with very dense material, embraces the basal part of the axoneme and mitochondrial derivatives which accompany almost the whole length of the axoneme (Pl. Ⅰ: 4).

The tail of spermatozoon appears oval in cross-section (Pl. Ⅰ: 5). The axoneme is a typical 9 + 9 + 2 pattern, in which the outer singlet tubules are hollow and connected by bilobate spots. Two broad bean-shaped accessory bodies are located lateral to the axoneme. Two mitochondrial derivatives almost completely crystallized, with only a small uncrystallized part at its center, are oval in cross-section. Two flat membranous cisterns, interposed between axoneme and mitochondrial derivatives, are S-shaped in cross-section. There are three granular connecting bands between mitochondrial derivatives and the axoneme, and the central one of them is ring-shaped (Pl. Ⅰ: 5). A character peculiar to this species, within the cytoplasmic membrane an additional bi-layer membrane which encircles the organelles (axoneme, mitochondrial derivatives, connecting bands, membranous cisterns and accessory bodies) of the tail, appears in some cross-sections of the tail. There was some very thin cytoplasm between the cytoplamic membrane and this additional membrane (Pl. Ⅰ: 6).

3 Discussion

The spermatozoon of *Mecopoda nipponensis* has the general characteristics of that of the Tettigonioidea described by Baccetti (1987): the laminar acrosomal complex lies lateral to the flat, dense nucleus and partially embraces it. In the typical 9 + 9 + 2 axoneme the outer singlet tubules are hollow and connected by bilobate spots. Two lateral additional fibrous structures (two accessory bodies in this species) are present. The usual three bands of granular material connecting the axoneme with the two mitochondrial derivatives, which are equally large and flanked by flat membranous cisterns. However, this species has some specialized characters. First, two mitochondrial derivatives are almost completely crystallized, not half crystallized as Baccetti described. Second, a character peculiar to this species, within the cytoplamic membrane an additional bi-layer membrane, which encircles organelles of the tail, appears in some cross-sections of the tail. In terms of the ultrastructure of the tail this seems to be a cross-section of the tail of the mature spermatozoon. However, this condition has never been found in spermatozoon of the other members in Tettigoniodea (Baccetti, 1987). In some stages of spermiogenesis in some species, such as *Conocephalus longipennis* (pers. obs.), there is a menbrane in stage 8 of the spermatid, but this does not appear to be the typical bi-layer membrane of the spermatozoon of *Mecopoda nipponensis*. In *M. nipponensis*, there is a bi-layer membrane besides the cytoplamic membrane, however, we are unsure whether this is an individual peculiarity of individual spermatozoa or a specialized transition morphologically from spermatid to mature spermatozoon, if it arises from the Golgi complex or other organelles and what role this membrane plays in the cell. More research is required to clarify the origin and role of this structure.

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日本纺织娘的精子超微结构
（直翅目：螽斯总科，纺织娘科）

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摘要：对日本纺织娘 *Mecopoda nipponensis* 的精子超微结构进行了透射电镜观察。结果表明：其顶体复合体侧生于细胞核上且包裹了核顶端的部分；细胞核致密，横切面多为新月形或半月形；2蚕豆形副体位于9+9+2轴丝的两侧；线粒体衍生体横切面为椭圆形，其基质几乎完全晶状化；线粒体衍生体一侧的2扁平膜池横切面近S形；轴丝与线粒体衍生体之间有3条连接带，其中中央的一条呈环状；在有些尾部横切面中，质膜之内又有一双层膜把尾部的细胞器围绕起来。

关键词：直翅目；纺织娘科；日本纺织娘；精子；超微结构

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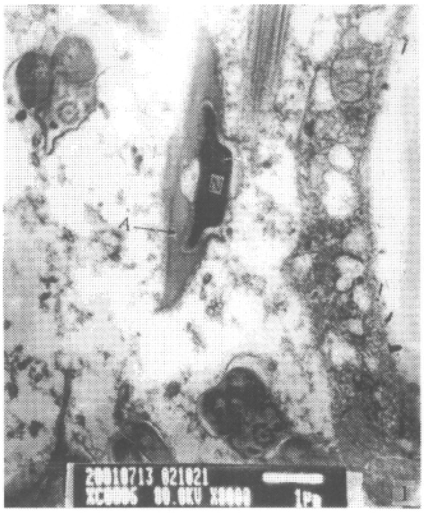

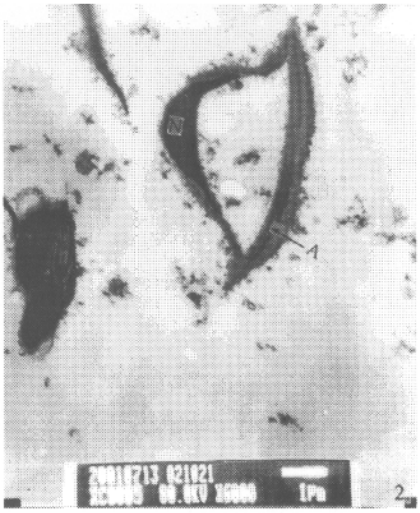

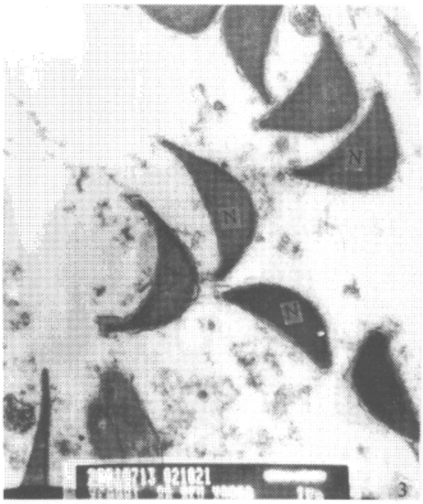

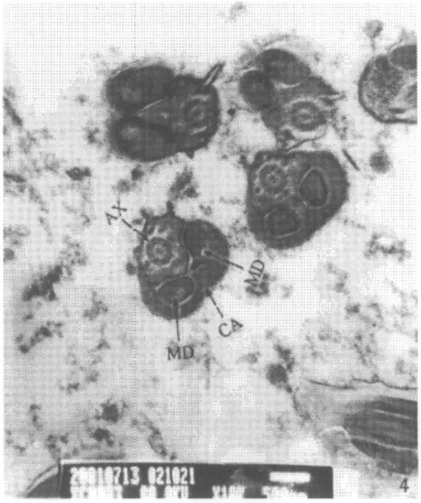

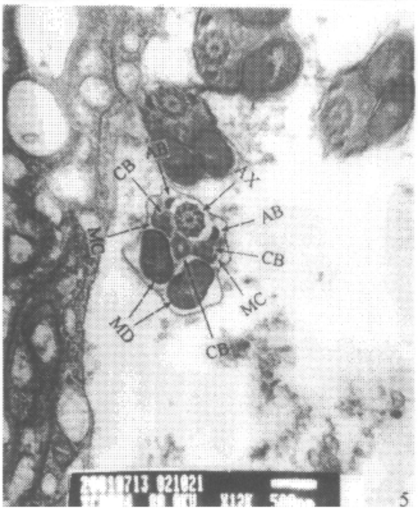

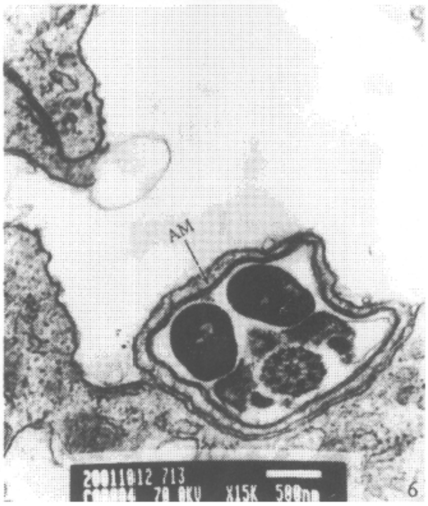

1. Cross-section of the head showing location of acrosomal complex and nucleus（精子头部横切面，示顶体复合体与细胞核）；2. Cross-section of the head showing location of acrosomal complex and nucleus（精子头部横切面，示顶体复合体与细胞核）；3. Cross-section of the head showing the shape of nucleus（精子头部横切面，示细胞核横切面）；4. Cross-section of the neck showing the centriolar adjunct（精子颈部横切面，示中心粒侧体）；5. Cross-section of the tail showing organelles of the tail（精子尾部横切面，示鞭毛各细胞器）；6. Cross-section of the tail showing the additional bi-layer membrane of the tail（精子尾部横切面，示细胞膜内的双层膜）；A：acrosome（顶体本体）；N：nucleus（核）；CA：centriolar adjunct（中心粒侧体）；AX：axoneme（轴丝）；MD：mitochondrial derivatives（线粒体衍生体）；MC：membranous cisterns（膜池）；AB：accessory bodies（副体）；CB：connecting bands（连接带）；AM：additional membrane（质膜内的双层膜）.